

POSITION PAPER

**SAFETY VALIDATION OF *GARCINIA CAMBOGIA* EXTRACT
(CITRIN[®] RANGE OF EXTRACTS, GARCITRIN[®])
AS A DIETARY SUPPLEMENT**

Prepared by
Sabinsa Corporation
70 Ethel Road West Unit 6
Piscataway, NJ 08854

May 21, 2009

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
1. INTRODUCTION	4
1.1. Background	4
1.2. Chemistry of Hydroxycitric Acid	5
1.3. Food Uses	6
2. SAFETY DATA	
2.1. Absorption, Metabolism, and Excretion	7
2.2. Mechanism of Action	7
2.3. Toxicological Studies	8
2.3.1. Acute and Short-term Studies	8
2.3.2. Subchronic Studies	9
2.3.3. Teratogenicity/Reproduction Studies	11
2.3.4. Genotoxicity	13
2.3.5. Human Studies	14
3. ANALYSIS OF ADVERSE EVENT REPORTS	19
4. CONCLUSIONS	22
5. REFERENCES	23
6. APPENDIX I	29

EXECUTIVE SUMMARY

On May 1, 2009, the FDA issued a warning to consumers, based on adverse event reports, to stop using Hydroxycut products by Iovate Health Sciences Inc., of Oakville, Ontario and distributed by Iovate Health Sciences USA Inc. of Blasdell, NY.

The active ingredients in an example product in the Hydroxycut line include *Garcinia cambogia* fruit rind extract (supplying calcium hydroxycitrate and potassium hydroxycitrate); Chromium polynicotinate; *Gymnema sylvestre* leaf extract; caffeine; Green tea extract (supplying polyphenols, catechins, 15% w/w epigallocatechin gallate (EGCG); White tea extract (supplying polyphenols, catechins, 15% w/w epigallocatechin gallate (EGCG); Oolong tea extract supplying polyphenols, catechins, 15%w/w epigallocatechin gallate (EGCG); Ginger extract (supplying gingerols); Raspberry ketone; and Quercetin dihydrate.

Although branded ingredients from Sabinsa Corporation do not occur in Hydroxycut, our scientific integrity dictates that we dispel any reservations on the safety of a healthful, natural, food ingredient, hydroxycitric acid, that forms a part of the active ingredients composition of Hydroxycut.

Based on the long history of food use of *Garcinia cambogia* fruits, the wealth of literature encompassing both preclinical and clinical studies, Sabinsa Corporation's clinical experience with Citrin® and 15 years of successful marketing of Citrin® extracts, there is no direct epidemiological or clinical evidence linking *Garcinia cambogia* fruit rind extract with hepatotoxicity. An exhaustive safety review by an independent panel of experts established GRAS affirmed status for Citrin®K, the potassium salt of hydroxycitric acid, in 2007. This paper reviews and analyzes the published safety documentation of *Garcinia cambogia* extract, its active component hydroxycitric acid and its salts.

Some evidence points to the hepatotoxicity of green tea polyphenols, represented by epigallocatechin gallate (EGCG), at levels much greater than those present in a cup of tea. Hepatotoxic effects of EGCG especially are more likely to be pronounced in a certain susceptible population. Hydroxycut contains extracts of green tea, white tea and oolong tea, all of which contain such polyphenols. Also published reports of chromium toxicity especially chromium polynicotinate in association with vegetable extracts are referred.

1. INTRODUCTION

1.1. Background

On May 1, 2009, the FDA issued a warning to consumers, based on adverse event reports, to stop using Hydroxycut products by Iovate Health Sciences Inc., of Oakville, Ontario and distributed by Iovate Health Sciences USA Inc. of Blasdell, N.Y. A copy of the statement and the supporting summary from the Health Hazard Evaluation Board, are available in Appendix 1.

An example of Hydroxycut product and its “Supplement Facts” panel is shown in Figure 1 (Ref: Health Hazard Evaluation Board Document, FDA). As seen in the supplement facts panel, the active ingredients include *Garcinia cambogia* fruit rind extract (supplying calcium hydroxycitrate and potassium hydroxycitrate); Chromium polynicotinate; *Gymnema sylvestre* leaf extract; caffeine; Green tea extract (supplying polyphenols, catechins, 15% w/w epigallocatechin gallate (EGCG); White tea extract (supplying polyphenols, catechins, 15% w/w epigallocatechin gallate (EGCG); Oolong tea extract supplying polyphenols, catechins, 15%w/w epigallocatechin gallate (EGCG); Ginger extract (supplying gingerols); Raspberry ketone (flavoring agent); and Quercetin dihydrate.

Although branded ingredients from Sabinsa Corporation do not occur in Hydroxycut, our scientific integrity dictates that we dispel any reservations on the safety of a healthful, natural, food ingredient, hydroxycitric acid, that forms a part of the active ingredients composition of Hydroxycut.

Garcinia cambogia is a small or medium sized tree native to Southeast Asia. The dried fruit rind of *G. cambogia* is commonly used, particularly in India, as a culinary flavoring agent, and a refreshing beverage is prepared from the fruit of a related variety *Garcinia indica*. Fruit from this plant is included in the United States Department of Agriculture’s inventory of perennial edible fruits of the tropics (Martin *et al.* 1987). The active ingredient of the fruit responsible for flavoring and other properties has been identified as (-)-hydroxycitric acid (HCA). The natural occurrence of HCA in the fruit was reported as early as 1883 by Lippman (Boll and Sorensen, 1969). Chemically, HCA is nearly identical to citric acid that gives citrus fruits their characteristic tart flavor. Currently, several products containing extracts from *G. cambogia* are marketed as dietary supplements.



Supplement Facts

Serving Size 2 Rapid Release Caplets
Servings Per Container 15

Amount Per Serving	% Daily Value
Calcium (as hydroxychloride)** 158 mg	10%
Chromium (as polynicotinate)** 133 mcg	111%
Potassium (as hydroxycitrate)** 218 mg	6%
Hydroxycut Plus® 1.32 g	
Garcinia cambogia extract (fruit)**	†
Standardized for 80% hydroxydictric acid	
Gymnema sylvestre extract (leaf)	†
Standardized for 25% gymnemic acids	
Phosphatidylserine-enriched soy lecithin	†
Supplying 50% phosphatidylserine, 4% phosphatidylcholine, 2% phosphatidylethanolamine	
HydroxyTea® 473 mg	
Green tea extract (as Camellia sinensis) (leaf)	†
Standardized for 90% polyphenols, 75% catechins, 45% epigallocatechin gallate - 111 mg EGCG	
Caffeine anhydrous	†
White tea extract (as Camellia sinensis) (leaf)	†
Standardized for 50% polyphenols, 35% catechins, 15% EGCG	
Oolong tea extract (as Camellia sinensis) (leaf)	†
Standardized for 80% polyphenols, 25% catechins, 15% EGCG	
Supplying 200 mg of caffeine	
Ginger extract (as Zingiber officinale) (root)	†
Standardized for 5% gingerols	
Raspberry ketone	†
Quercetin dihydrate (as Fava d'aria)	†

†Daily Value not established.

OTHER INGREDIENTS: MICROCRYSTALLINE CELLULOSE, HYDROXYPROPYLCELLULOSE, COATING (POLYVINYL ALCOHOL, TITANIUM DIOXIDE, POLYETHYLENE GLYCOL, TALC), SODIUM CARBONMETHYLCELLULOSE, CROSPONDONE, STEARIC ACID, MAGNESIUM STEARATE, SILICA, ACESULFAME-POTASSIUM.

The directions for the product are stated as follows:

For men and women:

Take 2 caplets with a glass of water 3 times daily, approximately 30 to 60 minutes before meals (preferably before breakfast, lunch and dinner). To assess individual tolerance, refer to the chart. Do not exceed 6 caplets in a 24-hour period. Do not take within 5 hours of bedtime. For best results, use Hydroxycut for 8 weeks in conjunction with a calorie-reduced diet and a regular exercise program. Do not snack after dinner. Consume ten glasses of water per day. Read the entire label before use and follow directions.

Figure 1: Hydroxycut: Example

1.2. Chemistry of Hydroxycitric Acid

The principal and active ingredient of Citrin® K, (-)-HCA (Figure 1), is chemically known as 1,2-dihydroxypropane-1,2,3-tricarboxylic acid. HCA has two asymmetric

centers and hence two pairs of diastereoisomers or four different optical isomers are possible. The diastereomers of HCA have been chemically synthesized from *cis* and *trans*-aconitic acid (Martius and Maue, 1941). (-)-HCA and (+)-HCA occur naturally in *Garcinia* and *Hibiscus* species (Lewis, 1969). Boll and Sorensen (1969) determined the absolute configuration of (-)-HCA employing Hudson's lactone rule, optical rotatory dispersion and circular dichroism curves and calculations of partial molar rotations. (-)-HCA possesses (S,S) configuration at both asymmetric centers.

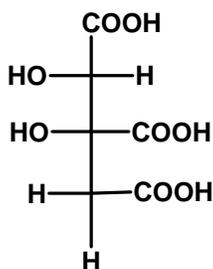


Figure 2. Chemical structures of (-)-Hydroxycitric acid ((-)-HCA)

In the dried fruit rind of *G. cambogia*, (-)-HCA is present at a level of 10-30% by weight. (-)-HCA can be isolated in the free form, as a mineral salt (*i.e.*, potassium-HCA, calcium-HCA, calcium/potassium-HCA, etc.) or as the lactone by several different methods. The free acid is unstable and is converted to its more stable lactone form. For consumer products, the free acid is often stabilized by forming salts of (-)-HCA (Majeed *et al.*, 1998).

1.3. Food Uses

The dried fruit rinds of *G. cambogia* plant are extensively used in South India for culinary purposes. The rinds are used as a condiment, in place of tamarind or lemon, for flavoring curries, meat and seafood dishes.

(-)-HCA, the active ingredient of *G. cambogia*, is a popular component of several dietary supplements marketed under different trade names. Commercially available *G. cambogia* extracts, prepared from the fruit rind, contain ~50% (-)-HCA (Jayaprakash and Sakariah, 2000; Jena *et al.*, 2002; Mattes and Bormann, 2000). Currently, more than 25 over-the-counter products marketed in the US have been reported to contain (-)-HCA.

2. SAFETY DATA

The safety of *G. cambogia* extract and its active ingredient (-)-HCA is supported by a variety of animal as well as *in vitro* experimental studies. Additional evidence from multiple human clinical trials further supports the safety aspects of (-)-HCA. Because of its potential health benefits, there has been considerable effort to elucidate the mechanism of action of (-)-HCA. Relevant biological and toxicological studies on *G. cambogia* extract and (-)-HCA supporting the safety in use of Citrin[®]K are included in the following sections. (Soni *et al.*, 2007)

2.1. Absorption, Metabolism, and Excretion

Since the publication of Heymsfield *et al.* (1998) indicating that oral administration of (-)-HCA to human subjects did not reduce body fat mass, the bioavailability of (-)-HCA has been the subject of debate (Badmaev *et al.*, 1999; Firenzuoli and Gori, 1999; Schaller, 1999). It has been suggested that bioavailability of (-)-HCA plays an important role in the efficacy of *G. cambogia* extract. In a recent human study, Preuss *et al.* (2005) reported that dosage levels, timing of administration and bioavailability are important factors for biological effects of (-)-HCA.

Loe *et al.* (2001) studied the bioavailability of calcium-potassium salt of (-)-HCA in four adult healthy human subjects (three males, one female). Subjects were given 2 g of (-)-HCA and following the ingestion, blood samples were collected every 30 minutes for up to 4 hours. Peak plasma HCA concentration ranged from 4.7 to 8.4 µg/ml. The plasma (-)-HCA concentration was found to range from 0.8 µg/ml at 30 minutes after ingestion to 8.4 µg/ml at 2 hours after ingestion. Plasma levels of (-)-HCA declined after 2.5 hours, but were considerably higher than the baseline level at 4 hours after ingestion. Presence of (-)-HCA was also detected in urine. These results suggest that (-)-HCA is readily absorbed in humans.

In another study, van Loon *et al.* (2000) investigated the acute effects of ingestion of *G. cambogia* extract on plasma HCA availability in human subjects. In this investigation, ten cyclists were monitored at rest and during 2 hours of exercise at 50% W_{max} on 2 occasions. At 45 and 15 minutes before exercise and 30 and 60 minutes after the start of exercise, subjects ingested 3.1 mL/kg body weight of an HCA solution (19 g/L) or placebo. In the pilot study, administration of a single bolus of HCA solution (4.4 g) over a 3.5 hour period resulted in peak HCA levels in plasma between 60 to 90 minutes after which the levels declined. In the main study, subjects received a total dose of 18 g HCA/person. Blood samples were collected at 15 minute intervals at rest and every 30 minutes during exercise. During resting period, after the ingestion of 4.4 g HCA, plasma HCA concentrations increased to 0.08 mmol/L (16.6 mg/L). Plasma HCA concentrations further increased to 0.39 mmol/L (82 mg/L) after the ingestion of 4.4 g HCA after 30 and 60 minutes of exercise. The results of this study clearly show that ingestion of large amounts of HCA results in increase in plasma HCA concentration. The amount of HCA ingested (18 ± 0.4 g) in the present study is 6 to 30 times higher compared to commonly recommended human doses.

2.2. Mechanism of Action

In the published literature different mechanisms of action of HCA have been proposed for the claimed anti-obesity effects *G. cambogia* extract and its active constituent. The primary mechanism of action of HCA has been reported to be competitive inhibition of the enzyme ATP-citrate lyase. This enzyme is known to catalyze the extramitochondrial conversion of citrate to oxaloacetate and acetyl coenzyme A (acetyl-CoA). Oxaloacetate may enter the gluconeogenic pathway leading to the production of glucose and glycogen. (Soni *et al.*, 2004).

In majority of the mechanistic studies, predominantly in rodents, HCA administration has been shown to inhibit lipogenesis in tissues responsible to convert carbohydrates to fatty acids, such as liver, adipose tissue, and small intestine. Additionally, HCA has been proposed to suppress feed intake *via* loss of appetite by stimulation of liver gluconeogenesis. Downs *et al.* (2005) suggested that HCA increases serotonin availability, reduces appetite, increases fat oxidation, improves blood lipid levels, reduces body weight, and modulates a number of obesity regulatory genes without affecting the mitochondrial and nuclear proteins required for normal biochemical and physiological functions.

2.3. Toxicological Studies

2.3.1 Acute and Short-term Studies

In an acute oral LD₅₀ study, gavage administration of *G. cambogia* extract [(-)-HCA 50% as calcium salt] to Crl:CD albino male and female rats (5/sex/group) at a dose level of up to 2500 mg/kg did not cause mortality. Clinical findings revealed soft stools and rales for one male and two females, respectively. No other adverse effects were noted (Ohia *et al.* 2002). The dermal LD₅₀ of *G. cambogia* extract in rabbits was reported as greater than 2000 mg/kg. *G. cambogia* extract was non-irritating to rabbits when given a single 500 mg dose was placed (semi-occluded) on shaved intact skin (Ohia *et al.*, 2002).

In an acute study, Louter-van de Haar *et al* (2005) investigated the effects of three different HCA-containing preparations (Regulator, Citrin[®]K, Super CitriMax HCA-600-SXS) on food intake and body weight in rats. Adult male Wistar rats were administered intragastrically 150 and 300 mg HCA/kg as a single dose or daily doses for four days. Following a single administration, no effect on body weight and water intake was observed in any of the experiments. At low dose, single administration did not affect food intake, but significant decrease in food intake was noted following administration of high dose Regulator and Citrin[®]K only. Both Regulator and Citrin[®]K significantly reduced cumulative food intake at days 1-4, whereas Super CitriMax HCA-600-SXS did not reduce food intake. Both Rregulator and Citrin[®]K resulted in a significantly lower gain of body weight compared to vehicle control.

In a short-term study, Sullivan *et al.* (1974b) investigated dose-related effects of sodium salt of HCA on lipid metabolism in Charles River SD strain female rats. Oral administration of 0.17 mmol/kg (~3 mg/kg) of HCA for 30 days resulted in decreased synthesis of cholesterol in the liver, adipose tissues and small intestine. At a dose level of about 11 mg/kg/day for 30 days, body weight gain was reduced without any changes in liver weight and liver lipid content. Administration of HCA at higher doses at levels up to 1350 mg/kg/day for 15 to 30 days resulted in reduced body weight gain and feed efficiency ratio (Sullivan *et al.*, 1974b; Chee *et al*, 1977; Rao and Sakariah, 1988).

In two separate experiments, Sprague Dawley male rats (6/group) were fed a diet containing 1 or 12% fat (Leonhardt and Langhans, 2002). Supplementation of both feeds with 3% HCA (~1500 mg/kg/day), after 10 days of restrictive feeding (10 g powdered standard rodent diet/day), reduced body weight regain and decreased the energy conversion ratio over the subsequent period of *ad-libitum* consumption (22 days). In rats

fed 12% fat diet, HCA reduced plasma triglycerol and increased liver fat concentration without affecting plasma β -hydroxybutyrate concentrations. The observation that HCA did not affect plasma β -hydroxybutyrate concentrations does not support the hypothesis that increased hepatic fatty acid oxidation is involved in the food intake suppression by HCA.

2.3.2 Subchronic Studies

In a well-designed 90-day oral toxicity study in male and female Sprague-Dawley rats, gavage administration of calcium potassium salt of (-)-HCA at dose level up to 2500 mg/kg/day did not induce any toxicologically significant effects (Shara *et al.* 2003a, b, 2004). In this study, rats were administered 0, 100, 1000 and 2500 mg/kg/day of *G. cambogia* extract containing calcium potassium salt of (-)-HCA, daily for 90 days. Daily examinations of rats for mortality/morbidity and clinical signs did not reveal any unusual findings. A significant reduction in feed consumption was noted at the end of 90 days (but not at 30 and 60 days) in both male and female rats given HCA. In both male and female rats, HCA administration also caused a significant reduction in body weight at the end of 60 and 90 days. At the end of 30, 60 and 90 days, blood samples were collected for clinical pathology. At these time points, rats were sacrificed and organ (adrenal, brain, heart, kidney, liver, prostate, spleen, testis and thymus) weights were recorded. The following organs were processed for histopathology: brain, pituitary, adrenal, eyes, trachea, esophagus, thyroid, heart, lung, salivary gland, liver, kidney, spleen, pancreas, stomach, intestine, ovaries, mammary glands, uterus, urinary bladder, testis and skin.

Hematological investigations at the end of 30, 60 and 90 days did not reveal any significant differences between the groups. Similarly, clinical chemistry parameters such as alkaline phosphatase, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, total bilirubin, glucose, calcium, chloride, phosphorus, sodium, potassium, iron, and total iron binding capacity did not reveal any significant differences between the groups at the end of 30, 60, or 90 days of treatment. Compared to controls, HCA treated groups did not reveal any statistically significant or biologically relevant differences in organ weights. Except minimal to mild changes in hepatocyte vacuolation, histological observations did not reveal any significant treatment-related effects in any of the organs studied.

The results of subchronic study reveal that oral administration of (-)-HCA at dose levels of 0, 100, 1000 and 2500 mg/kg/day to rats for 90 days did not induce any toxicologically significant alterations. The pathological changes in liver noted at the end of 30 and 60 days were not found in animals treated for 90 days. The results of this study suggest a no-observed-adverse-effect level (NOAEL) of *G. cambogia* extract for male and female rats of 2500 mg/kg/day (1500 mg HCA/kg/day). This study meets FDA core standards for safety assessment of food ingredients.

In another subchronic study of *G. cambogia* extract, six-week old male Zucker obese (fa/fa) rats were fed diets containing 0, 10, 51, 102 and 154 mmol HCA/kg diet (0, 77, 389, 778 and 1244 mg HCA/kg body weight/day) for 90 days (Saito *et al.*, 2005). Each diet group was pair-fed with the group that received the highest dose of HCA. The

highest dose group of animals receiving HCA-containing feed showed significant suppression of epididymal fat accumulations, compared with the other groups. Except testicular toxicity at two highest doses, no other adverse effects were noted. Obese rats maintained on feed containing 778 and 1244 mg HCA/kg/day showed testicular atrophy and toxicity, whereas diets containing 389 mg HCA/kg/day or less did not show any adverse effects. A critical review of this study revealed that the title of the article is misleading and there are some questions about the form of HCA (lactone form) used, the experimental design, and the suitability of Zucker obese rat for such a study.

According to Saito *et al.* (2005), the HCA content of the *G. cambogia* extract used was 41.2% by weight, and the ratio of its free-to-lactone form was 36.5 to 63.4. No information was provided as to the remaining 58.8% (by weight) contents of the extract. It is possible that the low free-to-lactone ratio, other constituents of the extract may be responsible for the noted toxicity at very high doses. In the above described Shara *et al.* (2003 a, b) subchronic study, readily water soluble, calcium-potassium salt of HCA containing 60% free form and 1% lactone form was used and investigators did not find testicular toxicity at dose levels up to 1500 mg HCA/kg/day. The difference in toxicity noted in these two studies may be related to the lactone content and lack of characterization of the extract in the Saito *et al.* (2005) study. Shara *et al.* (2003 a, b) did not find testicular toxicity as evidenced by weight of testis, testicular lipid peroxidation, DNA fragmentation, and histopathology at dose levels up to 1500 mg HCA/kg/day.

Hayamizu *et al.* (2008), given the testicular toxicity of HCA in animal models, investigated and concluded that ingestion of *G. Cambogia* extract at dose levels commonly recommended for human use does not affect serum sex hormone levels and blood parameters.

Secondly, Saito *et al.* (2005) reported that all groups in the 90-day study were pair fed with the highest dose group. It appears that the *ad libitum* fed controls were not included in the study. Animals maintained on the highest dose received 7% of *G. cambogia* extract (HCA, 3%) in their feed. Based on the amount of *G. cambogia* extract used in the rat diet and, as the groups were pair-fed to the highest dose, the amount of food received by pair-fed animals was approximately 10% less (compared to *ad libitum* fed control, which was not included). HCA is known to affect satiety, effectively preventing consumption of food at high levels. Toxicity testing of compounds (macro-ingredients) at >5% of the diet and substances that produce satiety preclude an interpretable outcome (Borzelleca, 1992). These factors may have affected the outcome of the study. Some dietary ingredients (methylxanthines, tea catechins) at high levels and dietary imbalances and nutritional restrictions are reported to cause testicular toxicity. It is not clear in the Saito *et al.* (2005) study, whether dietary imbalance or nutritional imbalance (use of high levels of the extract, pair feeding) may have contributed to the observed testicular toxicity at two high doses of *G. cambogia* extract employed.

Thirdly, Saito *et al.* (2005) stated that Zucker obese rats (or models with higher lipogenic properties) may be insensitive to HCA at usual dietary levels, however a biphasic effect of HCA on fat accumulation in the liver was noted. As presented in Table 4 of Saito *et al.* (2005) paper, at low doses of HCA treatment, fat accumulation was mild

to moderate, while at the highest dose, marked fat accumulation was noted. Interestingly, the fat accumulation at the highest dose was similar to that in the control. It is not clear how these changes may have affected the testicular toxicity. Additionally, the known powerful chelating action of free acid and lactone form of HCA may deplete zinc that is required for proper testicular development and function (Behne *et al.*, 1992). The free acid or lactone form of HCA appears to have sequestered enough zinc (or possibly iron or copper) in male rats to prevent proper testicular development and maintenance leading to testicular atrophy. Zinc deficiency is known to adversely affect testicular development and functions.

In a brief communication, Peal *et al.* (2007) investigated safety and efficacy of undenatured type II collagen (UCII) in combination with *G. cambogia* extract or chromium nicotinate in dogs. Although the objective of the study was to investigate the combination effects, one of the groups of dogs received only *G. cambogia* extract treatment. In this study, 25 dogs (average weight ~80 pounds or ~36 kg/dog) were divided in five groups (n=5) and each group received different treatment, *G. cambogia* extract alone and in combination with UCII, for a period of 120 days followed by a 30 day withdrawal. One of the groups received only *G. cambogia* extract treatment *via* oral route at a dose of 1800 mg/day for 120 days. The approximate dose of HCA in this study was determined as 30 mg HCA/kg/day. Compared to control group, animals treated with *G. cambogia* extract alone did not reveal any significant changes in body weight, body temperature, and serum chemistry related to liver and renal function (bilirubin, ALT, BUN, and creatinine) at 0, 30, 60, 90, 120, and 150 days. The results of this study suggest that treatment of dogs with *G. cambogia* extract at a daily dose of 1800 mg for 120 days did not cause any adverse effects.

In summary, daily administration of *G. cambogia* extract containing calcium potassium salt of HCA at dose levels up to 2500 mg/kg to rats for 90 days did not reveal any significant adverse effects. The results of another subchronic study of Zucker obese rats administered *G. cambogia* extract containing free form of HCA did not reveal any toxicity in female rats but resulted in testicular toxicity in males at high doses. Contrary to the testicular toxicity at high doses, histological observations of testis in the earlier study with higher levels of HCA did not reveal testicular toxicity or atrophy. It is likely that the testicular toxicity may vary, dependent on the lactone and salt content of HCA. Use of obese Zucker rats may not be appropriate for the study on testicular toxicity, as a defect in testicular testosterone production in these rats has been reported. Additionally lack of information on the extract (characterization) and questions on the design of the study, indicate that observations of testicular toxicity at high doses noted in this study may not be applicable to other forms of *G. cambogia* extract. In a study in dogs, administration of *G. cambogia* extract at a dose of 1800 mg/day for 120 did not reveal any adverse effects.

2.3.3 Teratogenicity/Reproduction Studies

In an unpublished study, Deshmukh (2007) investigated the effects of *G. cambogia* extract containing calcium/potassium salt of HCA on the reproductive systems of male and female rats, the postnatal maturation and reproductive capacity of their offspring, and

possible cumulative effects through multiple generations. In this study, Sprague Dawley rats (30/sex/group) were fed diet containing 0, 1000, 3000, or 10000 ppm of *G. cambogia* extract for 10 weeks prior to mating, during mating, and, for females through gestation and lactation, across two generations. During the course of the study, each rat was examined for signs of clinical toxicity, and body weight and feed intakes was recorded. For the parents (F₀ and F₁) and the offspring (F₁ and F_{2a}), reproductive parameters such as fertility and mating, gestation, parturition, litters, lactation, sexual maturity, and development of offspring were assessed. At termination, necropsy and histopathological examinations of organs were performed.

Exposure of parental male and female rats of both the generations (F₀ and F₁) during the pre-mating and mating periods, for both sexes, and during gestation and lactation in case of female rats to *G. cambogia* extract did not reveal any remarkable incidence of mortality or abnormal clinical signs. Compared to the respective control groups, exposure to *G. cambogia* extract did not affect feed consumption or body weight of rats at any of the exposure levels. Reproductive performance as evaluated by sexual maturity, fertility and mating, gestation, parturition, litter properties, lactation, and development of the offspring was not affected by feeding of diet containing *G. cambogia* extract. Based on the results of this study, the investigators concluded that the parental as well as the offspring NOAEL for *G. cambogia* extract was greater than 10000 ppm in diet or equivalent to 1018 and 1524 mg/kg body weight/day in male and female rats, respectively.

In the earlier described Saito *et al.* (2005) subchronic study, dietary exposure of Zucker rats to *G. cambogia* extract resulting in the intake of 778 and 1244 mg HCA/kg/day for 90 days caused testicular atrophy and impairment of spermatogenesis. Contrary to these observations, results from the two-generation reproductive toxicity study did not reveal any adverse effects of *G. cambogia* extract on testicular atrophy or spermatogenesis at dose levels up to 10000 ppm resulting in daily intake of 813 and 1018 mg/kg/day of the extract, in F₀ and F₁ generation of male rats, respectively. The resulting dose of HCA in this study was 488 and 611 mg HCA/kg/day. Compared to the Saito *et al.* (2005) study in which rats were exposed to *G. cambogia* extract for 90 days, the present study serves as a better model for observing the influences of *G. cambogia* extract on germ cell development, spermatogenesis, and sexual maturity. As against the results of Saito *et al.* (2005) study, the histopathological observations of rat testis from this study did not reveal any atrophy.

Additional circumstantial evidence also indicates that HCA, the active constituent of *G. cambogia* extract, is unlikely to cause developmental toxicity. In an earlier study, Greenwood *et al.* (1981) reported that feeding of female Zucker obese (*fa/fa*) rats with (-)-HCA containing diet for 39 days resulted in decrease in feed intake and body weight without affecting the percent of body fat. The (-)-HCA treated rats maintained a fat cell size equivalent to their obese controls. The observation that despite weight loss following (-)-HCA administration, obese rats still maintained fat cell size indicates that (-)-HCA is unlikely to affect fatty acid synthesis in either the maternal animals or their offspring. In an *in vitro* study, Jones and Ashton (1976) reported that (-)-HCA did not affect lipid synthesis in slices of fetal liver from guinea pigs. Additional evidence also suggests that

fatty acid synthesis pathways in fetal rat liver differ from those in adult rat liver (Watson *et al.*, 1969; Watson and Lowenstein, 1970; Jones and Ashton, 1976). Xu *et al.* (1990) reported that HCA exposure to 48 hour cultured tissue explants of 18-day fetal rat lung did not affect fatty acid synthesis stimulated by the hormone dexamethasone (known to stimulate the synthesis of fatty acids). These studies indicate that (-)-HCA does not affect fatty acid synthesis in the fetus.

In summary, the results of reproductive toxicity study suggest that *G. cambogia* extract is not a reproductive toxin in male and female rats. Based on the results of this study, the parental and offspring NOAEL was found to exceed 10000 ppm in diet or 1018 and 1524 mg/kg/day in male and female rats, respectively. Additional *in vitro* and *in vivo* observations indicate that (-)-HCA is unlikely to affect fatty acid synthesis in the fetus and support the premise that HCA is not a teratogen.

2.3.4 Genotoxicity

Mutagenic potentials of *G. cambogia* extract were investigated in an *in vitro* bacterial reverse mutation assay by plate incorporation and preincubation methods using five different strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) (Soni *et al.*, 2004). The concentrations of *G. cambogia* used in the plate incorporation assay were 52, 164, 512, 1600, and 5000 µg/plate. In this test, *G. cambogia* extract did not significantly increase the number of revertants in the presence or absence of metabolic activation. In the preincubation method, *G. cambogia* extract was tested at concentrations of 492, 878, 1568, 2800, and 5000 µg/plate. No statistically or biologically significant effects were noted in the number of revertants in strain TA98 and TA102 in the presence or absence of metabolic activation. In the strain TA100 and TA1535 without metabolic activation and in strain TA1537 with metabolic activation, significant increases were noted in the number of revertants. The tests were repeated number of times and it was concluded that these increases were not biologically indicative of mutagenic effect. The results of repeat experiments showed that increase was noted at the lowest or the alternate dose levels with no dose relationship, the statistical significance was considered as questionable. In strain TA102 with metabolic activation, precipitation was noted. The study concluded that *G. cambogia* extract was not mutagenic in the bacterial reverse mutation assay. As discussed below, in another study Lee and Lee (2007) reported that *G. cambogia* extract (60% HCA) did not cause mutagenic effects in *S. typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) in the presence or absence of metabolic activation.

Ono *et al.* (2006) investigated the *in vitro* and *in vivo* effects of *G. cambogia* extract (containing calcium salt of HCA) on induction of chromosome aberration in cultured Chinese hamster lung cells and the micronucleus test in mice. In the chromosome aberration test, Chinese hamster lung cells were exposed to *G. cambogia* extract at concentrations of 0, 125, 250, 500, 1000 and 2000 µg/ml for 6 to 24 hours in the presence or absence of microsomal activation. *G. cambogia* extract did not increase the number of cells with structural aberration and/or numerical aberrations. In the *in vivo* micronucleus test, male mice Slc:ddY were administered a single oral dose of 0, 500, 1000 or 2000 mg/kg *G. cambogia* extract and bone marrow cells were studied. No

significant increase in the frequency of micronucleated polychromatic erythrocytes (MNPCE) or ratio of polychromatic erythrocytes (PCE) to PCE plus normochromatic erythrocytes (NCE) was noted. These results show that *G. cambogia* extract does not induce chromosome aberration.

In another study, Lee and Lee (2007) investigated genotoxicity of HCA using a bacterial reverse mutation assay (Ames test), an *in vitro* chromosomal aberration test, and an *in vivo* micronucleus test. In the Ames test with *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537, *G. cambogia* extract (60% HCA) at concentrations up to 12.5 µmol/plate was negative in the presence or absence of a microsomal metabolizing system. Similarly, in the chromosomal aberration test, *G. cambogia* extract (12.5 µmol/ml) did not show significant mutagenic potentials. Oral administration of *G. cambogia* extract at doses of up to 2 g/kg did not induce MNPCE and the ratio PCE to PCE + NCE (Ono *et al.* 2006).

In summary, in Ames tests and in chromosomal aberrations with Chinese hamster lung cells, *G. cambogia* extract did not cause mutagenicity. In an *in vivo* micronucleus assay in mouse, *G. cambogia* extract at doses up to 2 g/kg did not cause any significant or dose related increases in the frequency of MNPCE. In another study in mouse, *G. cambogia* extract caused a dose dependent increase in MNPCE and the increase was only significant at 12500 µmol/kg *G. cambogia* extract (possibly 2.5 g/kg of HCA). There are some methodological and other questions as to the validity of the results noted. Given the structure of (-)-HCA and the available studies, (-)-HCA is unlikely to be genotoxic.

2.3.5 Human Studies

In the published literature, over 20 clinical trials with oral administration of *G. cambogia* extract to humans have appeared. The majority of the studies with *G. cambogia* extract involved overweight individuals. The objective of majority of these studies was to examine the effect of *G. cambogia* extract on weight reduction. Although the primary end point of majority of these investigations was to study the efficacy of *G. cambogia* extract, clinical observations also included any adverse effects. These studies provide an opportunity to assess the safety and 'tolerability' of *G. cambogia* extract in a diverse population. A summary of clinical trial design, doses and adverse effects noted in these investigations with oral *G. cambogia* extract treatment is given in Table 1.

Altogether, over 1000 subjects participated in these studies lasting for periods of up to 12 weeks. Of the 20 clinical trials found in the published literature, 17 studies have been identified as double-blind trials with over 800 participants. In majority of the clinical trials, the dosages of *G. cambogia* extract ranged from 1500 to 4667 mg/day (25 - 78 mg/kg/day). The equivalent (-)-HCA dose in these studies ranged from 900 - 2800 mg/day or 15 - 47 mg/kg/day. Clinical tolerance in these trials was evaluated by recording any adverse effects noted during the course of the trial. No adverse effects were noted or reported in the seven double-blind placebo-controlled trials. In other trials, some adverse effects were noted, but were not significantly different from the control group. Double-blind placebo-controlled trials are not considered to result in bias and collectively,

these trials on *G. cambogia* extract are of sufficient quality to draw some conclusion regarding the safety of the extract.

Some of the major clinical studies summarized in Table 1 are briefly discussed below. In a double-blind placebo-controlled trial over 135 subjects participated. In this study, *G. cambogia* extract (HCA) alone was used and subjects were randomized either to active (-)-HCA (*G. cambogia* extract) (n=66) or placebo (n=69). No overt signs of toxicity were observed among subjects consuming 1500 mg HCA/day (~15-18 mg HCA/kg/day) for 12 weeks. Forty two subjects from each group completed the study. The number of adverse reaction was not significantly different between the groups. The reported adverse effects in the treatment group included: headache (9), upper respiratory tract symptoms (16), and gastrointestinal symptoms (13); while in the placebo group adverse effects were headache (12), upper respiratory tract symptoms (13), gastrointestinal symptoms (6).

In six separate studies, Hayamizu and colleagues investigated effects of *G. cambogia* extract (Hayamizu *et al.*, 2001, 2002, 2003a, 2003b; Ishii *et al.*, 2003). Of the six trials, four were double-blind, placebo controlled involving 176 subjects. The dose of *G. cambogia* extract in these trials ranged from 1667 to 6000 mg/day and subjects were treated for up to 12 weeks. Of the two open label studies from this group, in one study, 2 subjects reported anorexia and one subject reported headache. In rest of the studies, including four double-blind, placebo-controlled studies, no treatment related adverse effects were noted.

In three separate double-blind, placebo-controlled trials by Preuss *et al.* (2002; 2004a, 2004b), efficacy and safety of *G. cambogia* extract was investigated. In these trials, 138 moderately obese subjects were given *G. cambogia* extract at a daily dose of 4667 mg (2800 mg HCA) alone or in combination with chromium nicotinate and *Gymnema sylvestre* for eight weeks. Changes in body weight, serum lipid profile, serum leptin levels, body mass index (BMI), urinary excretion of fat metabolites and appetite control were monitored. In addition to this hematology, serum clinical chemistry parameters and urinalysis were conducted. Administration of *G. cambogia* extract alone or combination of three ingredients resulted in significant weight loss, favorable lipid profile, reduction in BMI, decrease in leptin and increase in serotonin levels. Results of hematology, clinical chemistry and urinalysis did not reveal any treatment related changes. Compared to placebo group, administration of *G. cambogia* extract did not cause any significant adverse effects. These results along with other trials summarized in Table 1 suggest that administration of *G. cambogia* extract (HCA) at doses used in these studies does not cause any significant adverse effects.

Table 1. Reported adverse effects of *G. cambogia* extract in clinical trials

Reference	Study design	Number Subjects	Dose; Duration	Adverse Effects Reported
Badmaev and Majeed (1995)	Single arm, open label	77 enrolled; 55 completed	1500 mg GCE* and 300 µg chromium picolinate/day; healthy diet/exercise (8 weeks)	None reported
Conte (1993)	Randomized, double-blind, placebo-controlled	54 enrolled; 39 completed	1500 mg GCE and 300 µg chromium picolinate/day; healthy diet/exercise (8 weeks)	One reported itching around mouth
Girola <i>et al.</i> (1996)	Randomized, double-blind, placebo-controlled	150 enrolled; completed not reported	110 mg GCE and 38 mg chrom, 480 mg chitosan/day (or half all ingredient); hypocaloric diet (4 weeks)	None reported
Hayamizu <i>et al.</i> (2001)	Randomized, double-blind, placebo-controlled	40 enrolled; all completed	1667 mg GCE/day (8 weeks)	No adverse effects noted
Hayamizu <i>et al.</i> (2002)	Cross-over double blind, placebo-controlled	44 enrolled; all completed	6667 mg GCE/day (4000 mg HCA/day) (10 days)	No adverse effects noted
Hayamizu <i>et al.</i> (2002)	Open label	20 enrolled; 18 completed	5000 mg GCE/day (10 days)	No adverse effects noted
Hayamizu <i>et al.</i> (2003a)	Open label	10 enrolled; 10 completed	5000 mg GCE/day (30 days)	Anorexia 2 subjects and headache one subject
Hayamizu <i>et al.</i> (2003b)	Randomized, single-blind, placebo-controlled	44 enrolled; 39 completed	1667 mg GCE/day (12 weeks)	No treatment related adverse effects noted
Heymsfield <i>et al.</i> (1998)	Randomized, double-blind, placebo-controlled	135 enrolled; 84 completed	3000 mg GCE (1500 mg HCA)/day; high fiber low energy diet (12 weeks)	No treatment related adverse effects noted
Ishii <i>et al.</i> (2003)	Randomized, single-blind, placebo-controlled	48 enrolled; 46 completed	5000 mg GCE/day (12 weeks)	No treatment related adverse effects noted
Kovacs <i>et al.</i> (2001)	Randomized, double-blind, placebo-controlled	11 enrolled	500 mg HCA (alone or with 5 g medium chain triglyceride)/day(2 weeks)	No treatment related adverse effects noted
Kovacs <i>et al.</i> (2006)	Randomized, double-blind, placebo-controlled; cross over	10 enrolled; all completed	1447 mg HCA/day(10 days)	No treatment related adverse effects noted
Mattes and Bormann (2000)	Randomized, double-blind, placebo-controlled	89 enrolled	2400 mg GCE (1200 mg HCA)/day (12 weeks)	No adverse effects reported

Preuss <i>et al.</i> (2002)	Randomized, double-blind, placebo-controlled	48 enrolled	4667 mg GCE(2800 mg HCA)/day (8 weeks)	No adverse effects reported
Preuss <i>et al.</i> (2003a)	Randomized, single-blind, placebo-controlled	60 enrolled; 53 completed	4667 mg GCE(2800 mg HCA)/day (8 weeks)	No treatment related adverse effects noted
Preuss <i>et al.</i> (2003b)	Randomized, single-blind, placebo-controlled	30 enrolled; 29 completed	4667 mg GCE(2800 mg HCA)/day (8 weeks)	No adverse effects reported
Ramos <i>et al.</i> (1995)	Randomized, double-blind, placebo-controlled	40 enrolled; 35 completed	1500 mg GCE (8 weeks)	Two reported headache and nausea; one placebo reported similar symptoms
Rothacker <i>et al.</i> (1997)	Randomized, double-blind, placebo-controlled	50 enrolled; 48 completed	2400 mg GCE, 150 caffeine, 120 µg chromium/day; low fat diet (6 weeks)	None reported
Thom (1996)	Randomized, double-blind, placebo-controlled	60 enrolled	1320 mg HCA/day (low fat diet (8 weeks)	No adverse effects reported
Westerterp-Plantenga and Kovacs (2002)	Randomized, double-blind, placebo-controlled	24 enrolled; all completed	900 mg HCA/day (2 weeks)	No adverse effects reported
Antonio <i>et al.</i> (1999)	Parallel	20 enrolled, 18 completed	750 mg GCE, 750 mg Guggul extract, 750 mg calcium phosphate, 750 mg L-tyrosine; 7500 kJ/d diet plan (6 weeks)	No adverse events reported.
Thom (2000)	Parallel	40 enrolled, 40 completed	300 mg GCE, 1200 mg Phaseolus vulgaris Extract, and inulin; low fat 5000kJ/d diet	No adverse events reported

*GCE = *Garcinia cambogia* extract

In two separate pharmacokinetic studies in humans, oral administration of *G. cambogia* extract resulted in increase in (-)-HCA levels in blood suggesting that (-)-HCA was bioavailable in human plasma. (-)-HCA was absorbed from the intestine and the blood levels of (-)-HCA rapidly declined. The oral LD₅₀ of *G. cambogia* extract was reported as greater than 5000 mg/kg, suggesting that the extract is practically non-toxic.

Of the 20 clinical trials found in the published literature, 17 were placebo-controlled double-blind clinical trials and three were open label trials. As double-blind placebo-controlled trials are least likely to result in bias, these trials provide an

opportunity to assess safety. In some of these investigations, adverse effects were reported, but the incidence was similar to that of the placebo group. Majority of the adverse events were transient, minor and the frequency of adverse event was not related to the duration of the intake of *G. cambogia* extract. In two well designed trials where safety related parameters were investigated, use of *G. cambogia* extract/day at doses up to 4667 mg (2800 mg HCA/day or 78 mg HCA/kg/day for an individual weighing 60 kg) for up to 60 days did not result in any treatment-related adverse events. Similarly, in another clinical trial, administration of *G. cambogia* extract to human subjects at levels of 5000 mg/day for 12 weeks did not cause any adverse effects. Collectively, these trials suggest that ingestion of *G. cambogia* extract was well tolerated without any treatment-related adverse events.

In short and long term studies with a formulation containing Citrin®, a branded extract of *Garcinia cambogia* standardized to contain a minimum of 50% (-)HCA, in the form of the calcium salt (1500 mg corresponding to 750mg/day (-)HCA), no adverse events were noted. The duration of the study ranged from 8 to 36 months. The authors reiterate that long term studies the (-)HCA formula did not produce any objective and subjective side effects (Badmaev *et al*, 2002). Also, the therapy did not affect the vital signs of treated individuals, i.e. the heart rate and blood pressure parameters. Blood chemistry data obtained prior to the (-)HCA formula regimen, after eight weeks and up to three years of the continuous regimen, indicate that no significant difference in any biochemical parameters related to the function of major organs or systems in the body occurred. Patients for the long-term study were recruited from the same pool of ambulatory patients who participated in the short-term study. The overweight subjects evaluated long term were 12 women and one man, 33 to 76 years old, and were carefully selected to comply with the guidelines of the long term study.

The several months intake of the (-)HCA formula also resulted in a significant ($p<0.05$) weight reduction from a mean value of 158 (CI: 139-177) lbs. to a mean value of 146 lbs. The blood lipid profile evaluated before and after the long-term treatment showed a pattern similar to that seen in the eight-week study, however numerical changes were not statistically significant. On the other hand, the mean value for CHD risk index in the studied population was 0.94 before and 0.87 after the treatment, and this change was statistically significant ($p<0.05$). In the long-term study, most participants had health problems. Twelve of the 13 long term patients had preexisting medical conditions, i.e. dyslipidemia (5/13), essential hypertension (3/13), hormonal imbalance (3/13), mental depression (2/13), Crohn's disease (1/13), hypothyroidism (1/13), epilepsy (Petit mal) (1/13), osteoarthritis (1/13), allergic sinusitis (1/13), reflux esophagitis (1/13), and gout (1/13). The patients received the following prescription medications concomitant with the administration of the (-)HCA formula: Inderal, Lotensin, Questram, hydrochlorothiazide, Estraderm patch, Prozac, Librium, Prilosec, Zantac, Tavist D, Benemid, Prinivil, allopurinol, Motrin, Depakote, Tegretol, Neurontin. During the eight week study, and up to three years of continuous intake of the (-)HCA formula, no side effects nor drug incompatibility were reported by the patients or their supervising physician. (Badmaev, *et al*, 2002).

The clinical evidence of *G. cambogia* extract safety is supported by the following facts:

- Majority of the animal toxicity studies of *G. cambogia* extract are conducted with products containing salts of (-)-HCA.
- The fruit from which *G. cambogia* extract is prepared is commonly used as a culinary ingredient in South and Southeast Asia.
- The principal constituent of the extract, (-)-HCA is structurally similar to citric acid an endogenous substance found in human body.
- In multiple human clinical studies, safety of the extract was confirmed at doses up to 4667 mg/day or higher.
- There is no evidence that consumption of *G. cambogia* extract either in foods or as a dietary supplement has a cumulative effect that would affect its safety.
- A variety of animal and *in vitro* studies with salts of *G. cambogia* extract corroborate the human clinical safety data.
- Reproduction toxicity study in rats did not reveal any adverse effects of *G. cambogia* extract, thus negating the testicular toxicity noted in a study in obese rats.

In a systematic review of adverse events associated with herbal weight loss supplements, Pittler & Ernst (2004) & Pittler *et al* (2005) concluded that “*Garcinia cambogia* extract has been tested in a number of trials. These trials and case reports detail few and mild adverse events for both *Garcinia cambogia* and hydroxycitric acid”. They reported adverse events associated with most herbal weight management support ingredients at that time, and concluded that only *Garcinia cambogia* and Yerba mate merited further consideration as weight management support ingredients, on account of their safety.

3. ANALYSIS OF “ADVERSE EVENT” REPORTS

Dietary supplements containing (-) HCA, *Garcinia cambogia* extract, have been marketed in the US since 1994. Millions of doses containing the extract have been sold, with no adverse event reports related specifically to the ingredient. Any adverse event reports appearing in the medical literature involved mixed formulations.

The recent literature reports of hepatitis cases linked with use of botanical food supplement Hydroxycut require careful analysis, especially since these reports provided grounds for the FDA recall of the supplement. In one publication (Dara *et al*, 2008) two cases of hepatitis were described with different clinical and laboratory pictures, i.e. a 40 year old female, body builder, with gastrointestinal discomfort accompanied by elevated liver enzymes, and 33 year old female presenting with 1 month of new-onset jaundice, gastrointestinal discomfort, elevated liver enzymes and increase in anti-nuclear antibody (ANA) and anti-smooth muscle antibody (ASMA) titers suggestive of autoimmune process. In both cases symptoms resolved with liver function tests returning to normal.

In case of the first patient, the body builder, the differential diagnosis should also include evaluation for use of anabolic steroids. The use of anabolic steroids among athletes and body builders is widespread, and for obvious reasons often not reported by

the patients. Numerous reports have noted liver dysfunction secondary to anabolic steroid use presenting with jaundice and elevated serum aminotransferase levels (Štimac *et al*, 2002; Pertusi *et al*, 2001). In the second patient differential diagnosis should include hepatitis E in view of patient reporting eating lobster prior to illness. Hepatitis E virus is increasingly a cause of waterborne and foodborne hepatitis in underdeveloped but also in industrialized countries. Laboratory tests in hepatitis E may demonstrate positive anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA) and high level of serum immunoglobulin G (IgG) (Nagasaki *et al*, 2005).

The reports of hepatotoxicity possibly linked to use of Hydroxycut were described in 27 year old, 30 year old (Stevens *et al*, 2005) and 19 year old men respectively (Jones and Andrew, 2007). The last patient was a soldier stationed in Iraq. The three patients underwent an extensive diagnostic blood workup which did not include evaluation for use of anabolic steroids and hepatitis E. Interestingly the symptoms of liver injury occurred in relation to few days to several months intake of Hydroxycut. This discrepancy maybe relevant in the discussion Hydroxycut as a potential causative agent for the reported cases of hepatitis. In all three patients the clinical symptoms and laboratory indices normalized within 4 weeks to 4 months after the onset of illness.

The authors of these reports involving 5 patients attempted to analyze the ingredients of multicomponent Hydroxycut in relation to the potential hepatotoxicity. Based on previously published papers the authors of first report linked hepatotoxicity with use of *Garcinia cambogia* fruit extract, chromium and *Camelia Sinensis* (tea extract). The shortcomings of this report should be pointed out. *Garcinia cambogia* is traditionally used as food and spice, and not as reported by the authors “to make meals more filling”. The authors also confuse toxicity of known hepatotoxin monteleukast with action of *Garcinia cambogia* extract. The authors state that “A MEDLINE search did not reveal previous cases of hepatotoxicity resulting from *Garcinia cambogia*, *Gymnema sylvestre*, willow bark, glucomannan, green tea or guarana extract”. In fact there are several references related to potential hepatotoxicity due to tea extracts and salicylates (willow bark). On the other hand the entry “Hydroxycitric acid hepatotoxicity” or “*Garcinia cambogia* hepatotoxicity” in MEDLINE does not yield references. The authors of the third report attribute potential hepatotoxicity of Hydroxycut to *Camelia sinensis* (tea) extract providing two references on potential hepatotoxicity of green tea and hydroalcoholic extract of green tea.

In summary, there are three short communications on potential hepatotoxicity of Hydroxycut in the medical literature reporting 5 cases, 2 young women and 3 young men. The analysis of reported clinical and laboratory findings in 5 patients revealed that differential diagnosis did not rule out liver injury due to anabolic steroids use and other causes of hepatitis, e.g. hepatitis E. It should also be noted that majority of health professionals are lacking knowledge of nutritional supplements that are increasingly popular in general population and has become a permanent part of practicing medicine in this country.

In another report a patient receiving chronic treatment with a leukotriene receptor antagonist in conjunction with self prescribed supplements for weight reduction.

developed fatal liver failure. Prior to the occurrence of the adverse event, the patient had taken two dietary supplements, for weight reduction for 7 days. These supplements supplied several active ingredients: Hydrolyzed collagen, *Gymnema Sylvestre*, Sodium hyaluronate, *Garcinia cambogia*, *Betula alba* leaf extract, Bean Pod, *Citrus Aurantium* leaf extract, *Magnolia Officinalis* bark extract, Chromium, *Bambusa vulgaris* leaf extract, Carnitine, Glutamine, Phenylalanine, L-Ornithine, Magnesium pyruvate. The authors could only make speculative conclusions on the potential drug interactions that had occurred, as there was no epidemiological history or prior clinical evidence. Montelukast, which the patient had assumed for 5 years, belongs to the class of the leukotriene receptor antagonists. A long latency in the appearance of the liver necrosis, ranging between 2 and 18 months up to 2 years, and the capacity to induce multilobular necrosis requiring liver transplant marked the toxicity pattern of this class of drugs. The authors also mentioned that the supplements contained *Citrus aurantium* whose capacity to interfere with cytochrome functions has been extensively studied. Two herbal supplements were suspected to exacerbate the effects of hepatotoxic montelukast; one of them contained *Garcinia cambogia* whereas both the supplements contained *Citrus aurantium*. [(Actis et al, 2007).

Shim and Saab (2009) described a case of a 28 year old man taking Hydroxycut as per the manufacturer's recommended dose and concurrently taking a pain relieving medicine containing acetaminophen 250mg, aspirin 250mg and caffeine 65 mg, four tablets per day for the 10 days leading to the development of symptoms of 3 weeks of fatigue, dyspnea on exertion, jaundice and dark urine. The authors could not however exclude the interaction between the compounds of Hydroxycut, and acetaminophen which the patient was taking concurrently.

Chromium, an essential trace element and cofactor to insulin, is most commonly occurring in hexavalent (VI, associated with occupational toxicity in the leather industry) and trivalent (III) states (the ingested form). Each Hydroxycut serving contains 133 micro gm of Chromium, which is taken three times daily, resulting in a cumulative daily consumption which is twice the maximum safe daily dose (as recommended by the National Academy of Science).

Chromium has been reported be responsible for hepatotoxicity when ingested with vegetable extracts. A clinical case of acute hepatitis in a patient undergoing an alternative medicine weight-reduction regimen has been reported. Chromium polynicotinate had been ingested in combination with vegetable extracts over a 5-month period. Liver biopsy was compatible with toxic hepatitis and greatly elevated hepatic chromium levels were found (>10× normal). The clinical picture regressed following suspension of the medication (Lanca *et al*, 2002)

Camellia sinensis (green tea) has been associated with hepatotoxicity in published reports. A recent review (Mazzanti *et al*, 2009) that summarized a literature search of publications between 1999 and October 2008 retrieved 34 cases of hepatitis, associated with green tea extract consumption. Histological examination of the liver revealed inflammatory reactions, cholestasis, occasional steatosis, and necrosis. A positive dechallenge was reported in 29 cases. There was one reported death. A positive

rechallenge occurred in seven cases (20%). Toxic effects tend to arise when people take green tea supplements, which can contain more than 50 times as much polyphenol as a single cup of tea (Mead, 2007). Data available suggests that high doses of (-) Epigallocatechin gallate (EGCG) can induce toxicity in the liver, kidney and intestine. Toxicity, especially in the liver and kidney, appears to be correlated with the bioavailability of EGCG (Lambert *et al*, 2007). Laboratory research with both rodents and dogs has shown that high doses of the most heavily studied green tea polyphenol, (-)-epigallocatechin-3-gallate (EGCG), cause liver, kidney, and gastrointestinal toxicities (Lambert *et al*, 2007). Recent studies in human have also demonstrated that fasting increases the bioavailability of EGCG (Chow *et al*, 2005). Ethanolic extract of *C. sinensis* has been banned in France and Spain (WHO Newsletter)

Thermogenesis-induced weight loss produced by green tea's epigallocatechin gallate and its ability to inhibit catechol-*O*-methyltransferase (COMT) is important for health benefits and for prolonging the action of norepinephrine in the synaptic cleft (Hydroxycut hard-core FAQs). One review postulates that some susceptible individuals have a polymorphism of a gene coding for COMT, a key enzyme in protecting cells against EGCG-mediated oxidative stress and hepatotoxicity. About a quarter of the population have a polymorphism that is associated with low activity of COMT which increases exposure to the unmetabolized parent compound resulting in the observed liver toxicity. (Mead 2007)

Gymnema sylvestre has a history of thousands of years of use as a taste modifier (the active constituents bind to sweetness receptors on the tongue) and medicinal herb in supporting the maintenance of healthy blood sugar levels. A number of documented clinical studies report no adverse events, associated with its use. (Yeh *et al*, 2003)

4. CONCLUSIONS

Some evidence points to the hepatotoxicity of green tea polyphenols, represented by epigallocatechin gallate (EGCG), at levels much greater than those present in a cup of tea. Hydroxycut contains extracts of green tea, white tea and oolong tea, all of which contain such polyphenols. Chromium polynicotinate, another ingredient of Hydroxycut®, has been reported to cause hepatotoxicity especially in association with vegetable extracts.

It appears that based on the available literature, there are no studies or epidemiological evidence to implicate *Garcinia cambogia* in the hepatotoxicity of Hydroxycut.

5. REFERENCES

1. Actis, G. C., Bugianesi, E., Ottobrelli, A., Rizzetto, M., 2007, Fatal liver failure following food supplements during chronic treatment with montelukast, *Digestive and Liver Disease*, 39, 953
2. Lobb, A., 2009. Hepatotoxicity associated with weight-loss supplements: A case for better post-marketing surveillance, *World J Gastroenterol.*, 15,1786-87
3. Antonio J, Colker CM, Torina GC, Shi Q, Brink W, Kalman D. Effects of a standardized guggulsterone phosphate supplement on body composition in overweight adults: a pilot study. *Curr Ther Res* 1999; 60:220–7.
4. Dara, L., Hewett, J., Lim, K. J., 2008, Hydroxycut hepatotoxicity: A case series and review of liver toxicity from herbal weight loss supplements, *World J Gastroenterol.*, 14, 6999-7004
5. Badmaev, V., Majeed, M., Conte, A.A., 1999. *Garcinia cambogia* for weight loss. *JAMA* 282, 233-235.
6. Badmaev, V., Majeed, M., 1995. Open field, physician controlled, clinical evaluation of botanical weight loss formula Citrin. Presented at Nutracon 1995: Nutraceuticals, Dietary Supplements and Functional Foods. Las Vegas, Nevada, July 11-13
7. Badmaev, V, Majeed, M, Conte, A. 2002 Open field, physician controlled clinical evaluation of a botanical weight loss formula based on *Garcinia cambogia* derived (-) hydroxycitric acid. *Nutracos*, Jan-Feb 11-14.
8. Behne, D., Kyriakopoulos, A., Gessner, H., Vormann, J., Gunther, T., 1992. Sex-related effects of zinc deficiency on the selenium metabolism in rats. *Journal of Trace Elements and Electrolytes in Health and Disease* 6:21-25.
9. Boll, P.M., Sorensen, E., Balieu, E., 1969. Naturally occurring lactones and lactams. The absolute configuration of the hydroxycitric acid lactones: Hibiscus acid and *Garcinia* acid. *Acta Chemica Scandinavica* 23, 286-293.
10. Borzelleca, J.F., 1992. Macronutrient substitutes: safety evaluation. *Regulatory Toxicology and Pharmacology* 16, 253-264.
11. Chee, H., Romosos, D.R., Leveille, G.A., 1977. Influence of (-)-hydroxycitrate on lipogenesis in chickens and rats. *Journal of Nutrition* 107, 112-119.
12. Chow, H. H., Hakim, I. A., Vining, D. R., Crowell, J. A., Ranger-Moore, J., Chew, W. M., Celaya, C. A., Rodney, S. R., Hara, Y., Alberts, D. ., 2005. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of polyphenon E in healthy individuals, *Clin. Cancer Re.*, 11, 4627-4633
13. Conte, A.A., 1993. A non-prescription alternative in weight reduction therapy. *American Journal of Bariatric Medicine* Summer, 17-19.
14. Deshmukh, N.S., 2007. Two-Generation Reproduction Toxicity Study of Super CitriMax in Sprague Dawley Rats. Study No. 4493. Report No. IR/4493/RT-DT-R/06. INTOX PVT. LTD. 375, Urawade, Tal. Mulshi, Dist. Pune – 412 108 Maharashtra, India.
15. Downs, B.W., Bagchi, M., Subbaraju, G.V., Shara, M.A., Preuss, H.G., Bagchi, D., 2005. Bioefficacy of a novel calcium-potassium salt of (-)-hydroxycitric acid. *Mutation Research* 579, 149-62.
16. DSHEA, 1994. Dietary Supplements Health and Education Act of 1994. US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC. Available at website: <http://vm.cfsan.fda.gov/~dms/dietsupp.html>
17. Firenzuoli, F., Gori, L., 1999. *Garcinia cambogia* for weight loss. *JAMA* 281, 234, Author reply 235.

18. Girolo, M., De Bernardi, M., Contos, S., 1996. dose effect in lipid-lowering activity of a new dietaryintegrator (Chitosan, *Garcinia cambogia* extract, and Chrome). *Acta Toxicologica et Therapeutica* 17, 25-40.
19. Greenwood, M.R., Cleary, M.P., Gruen, R., Blasé, D., Stern, J.S., Triscari, J., Sullivan, A.C., 1981. Effect of (-)-hydroxycitrate on development of obesity in the Zucker obese rat. *American Journal of Physiology* 240, E72-E78.
20. Hayamizu, K., Ishii, Y., Kaneko, I., Shen, M., Sakaguchi, H., Okuhara, Y., Shigematsu, N., Miyazaki, S., Shimasaki, H., 2001. Effects of long-term administration of *Garcinia cambogia* extract on visceral fat accumulation in humans: a placebo-controlled double blind trial. *Journal of Oleo Science*, 50, 805-812.
21. Hayamizu, K., Ishii, Y., Kaneko, I., Shen, M., Okuhara, Y., Sakaguchi, H., Shigematsu, N., Shimasaki, H., 2002. No-observed-adverse-effect level (NOAEL) and sequential-high-doses administration study on *Garcinia cambogia* extract in humans. *Journal of Oleo Science* 51, 365-369.
22. Hayamizu, K., Ishii, Y., Shigematsu, N., Okuhara, Y., Tomi, H., Furuse, M., Yoshino, G., Shimasaki, H., 2003a. Safety of *Garcinia cambogia* extract in healthy men: high-doses administration study I. *Journal of Oleo Science* 52, 499-504
23. Hayamizu, K., Ishii, Y., Kaneko, I., Shen, M., Okuhara, Y., Shigematsu, N., Tomi, H., Furuse, M., Yoshino, G., Shimasaki, H., 2003b. Effects of *Garcinia cambogia* (hydroxycitric acid) on visceral fat accumulation in humans: A double-blind, randomized, placebo-controlled trial. *Current Therapeutic Research* 64, 551-567.
24. Hayamizu, K., Tomi, H., Kaneko, I., Shen, M., Soni, M. G., Yoshino, G. 2008. *Fitoterapia*, 79, 255-261
25. Hayashi, M., Tice, R. R., MacGregor, J. T., Anderson, D., Blakey, D. H., Kirsh-Volders, M., Oleson, F. B. Jr., Pacchierotti, F., Romagna, F., Shimada, H., 1994. *In vivo* rodent erythrocyte micronucleus assay. *Mutation Research* 312, 293-304.
26. Heymsfield, S.B., Allison, D.B., Vasselli, J.R., Pietrobelli, A., Greenfield, D., Nunez, C., 1998. *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. *JAMA* 280, 1596-1600.
27. Hydroxycut Hardcore FAQs <http://www.hydroxycuthardcore.us/FAQ.html>
28. Ishii, Y., Kaneko, I., Shen, M., Hayamizu, K., Shigematsu, N., Tomi, H., Yoshino, G., Shimasaki, H., 2003. Safety of *Garcinia cambogia* Extract in Healthy Volunteers: High-Dose Administration Study II. *Journal of Oleo Science* 52, 663-671.
29. Jayaprakasha, G. K., Sakariah, K. K., 2000. Determination of (-)Hydroxycitric acid in commercial samples of *Garcinia cambogia* extract by liquid chromatography with ultraviolet detection , *J Liquid Chrom. & Related Technologies* 23, 915-23
30. Jena, B.S., Jayaprakasha, G.K., Singh, R.P., Sakariah, K.K., 2002. Chemistry and biochemistry of (-)-hydroxycitric acid in commercial samples *J Agri. Biol. Chem.*, 50, 10-22
31. Jones, C.T., Ashton, I.K., 1976. Lipid biosynthesis in liver slices of the foetal guinea pig. *Biochemistry Journal* 154, 149-158.
32. Jones, F. J., Andrew, A. H., 2007. Acute liver injury associated with the herbal supplement hydroxycut in a soldier deployed in Iraq. *Am. J Gastroenterol.*, 102, 2357
33. Kovacs, E.M., Westerterp-Plantenga, M.S., de Vries, M., Brouns, F., Saris, W.H., 2001. Effects of 2-week ingestion of (-)-hydroxycitrate and (-)-hydroxycitrate combined with medium-chain triglycerides on satiety and food intake. *Physiology and Behavior* 74, 543-549.
34. Kovacs, E.M., Westerterp-Plantenga, M.S., 2006. Effects of (-)-hydroxycitrate on net fat synthesis as de novo lipogenesis. *Physiology and Behavior* 88, 371-381.

35. Lambert, J. D., Sang, S., Yang, C. S., 2007, Possible Controversy over Dietary polyphenols: Benefits vs Risks. *Chemical Research in Toxicology*, 20, 583-585
36. Lanca, S., Alves, A., Vieira, A. I., Barata, J., de Freitas, J., de Carvalho, A., 2002, *European J Internal Medicine.*, 13, 518-20
37. Louter-van de Haar, J., Wielinga, P.Y., Scheurink, A.J., Nieuwenhuizen, A.G.. 2005. Comparison of the effects of three different (-)-hydroxycitric acid preparations on food intake in rats. *Nutrition and Metabolism* 2, 23.
38. Lee, K.H., Lee, B.M., 2007. Evaluation of the genotoxicity of (-)-hydroxycitric acid (HCA-SX) isolated from *Garcinia cambogia*. *Journal of Toxicology and Environmental Health A* 70, 388-392; Comment 2008, 71, 348-349; Author Reply 350-351.
39. Lewis, Y.S., 1969. Isolation and properties of hydroxycitric acid. In: Lowenstein, J.M. (Ed.), *Methods in Enzymology*. In: *Citric Acid Cycle*, Vol. 13. Academic Press, New York , NY, pp. 613-619.
40. Loe, Y.C., Bergeron, N., Rodriguez, N., Schwarz, J.M., 2001. Gas chromatography/mass spectrometry method to quantify blood hydroxycitrate concentration. *Analytical Biochemistry* 292, 148-154.
41. Leonhardt, M., Langhans, W., 2002. Hydroxycitrate has long-term effect on feeding behavior, body weight regain and metabolism after body weight loss in male rats. *Journal of Nutrition* 132, 1977-1982.
42. Majeed, M., Badmaev, V. and Rajendran, R., 1998. Potassium hydroxycitrate for the suppression of appetite and induction of weight loss. Assignee: Sabinsa Corporation (Piscataway, NJ). US Patent # 5,783,603. March 31, 1997. US Patent and Trademark Office, Washington DC.
43. Martin, F.W., Campbell, C.W., Ruberte, R.M., 1987. Perennial edible fruits of the tropics: an inventory. United States Department of Agriculture, Agricultural Research Service, Washington, DC, p. 212.
44. Martius, C., Maue, R., 1941. Preparation, physiological behavior, and importance of hydroxycitric acid and its isomers. *Zeitschrift fur Physiological Chemistry* 269, 33-39.
45. Mattes, R. D., Bormann, L., 2000. Effects of (-)-hydroxycitric acid on appetitive variables. *Physiology and Behavior* 71, 87-94.
46. Mazzanti, G., Menniti-Ippolito, F., Moro, P. A., Casseti, F., Raschetti, R., Santuccio, C., Mastrangelo, S., 2009. Hepatotoxicity from green tea: a review of the literature and two unpublished cases 65, 331-341
47. Mead, M. N., 2007, Temperance in Green Tea, *Environmental Health Perspectives*. 115 (9), A445 quoting Prof. C. S. Yang (Rutgers University).
48. Nagasaki, F; Ueno, Y; Mano, Y; Igarashi, T; Yahagi, K; Niitsuma,H; Okamoto H; and Shimosegawa, . 2005. Case Report: A Patient with Clinical Features of Acute Hepatitis E Viral Infection and Autoimmune Hepatitis. *The Tohoku Journal of Experimental Medicine* Vol. 206 , No. 2 pp.173-179

49. Ohia, S.E., Opere, C.A., LeDay, A.M., Bagchi, M., Bagchi, D., Stohs, S.J., 2002. Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). *Molecular and Cellular Biochemistry* 238, 89-103.
50. Ono, H., Tamura, H., Yamashita, Y., Tamura, K., Iwakura, K., 2006. *In vitro* chromosome aberration test and *in vivo* micronucleus test of Ca-type *Garcinia* extract. *Shokuhin Eiseigaku Zasshi* 47, 80-84.
51. Peal, A., D'Altilio, M., Simms, C., Alvey, M., Gupta, R.C., Goad, J.T., Canerdy, T.D., Bagchi, M., Bagchi, D., 2007. Therapeutic efficacy and safety of undenatured type-II collagen (UC-II) alone or in combination with (-)-hydroxycitric acid and chondroitin in arthritic dogs. *Journal of Veterinary Pharmacology and Therapeutics* 30, 275-278.
52. Pertusi, R; Dickerman, RD; McConathy, WJ . 2001. Evaluation of aminotransferase elevations in a bodybuilder using anabolic steroids: hepatitis or rhabdomyolysis? *JAOA* 101(7) , 391-394
53. Pittler, M. H., Ernst, E., 2004. Dietary supplements for body-weight reduction: a systematic review, *Amer J Clin Nutrition*, 79, 529-36
54. Pittler, M. H., Schmidt, K., Ernst, E, 2005. Adverse Events of herbal supplements for body weight reduction: systematic review. *Obesity Reviews* 6, 93-111
55. Preuss, H.G., Bagchi, D., Rao, C.V.S., Echard, B.W., Satyanarayana, S., Bagchi, M., 2002. Effect of hydroxycitric acid on weight loss, body mass index and plasma leptin levels in human subjects. *FASEB Journal* 16, A1020.
56. Preuss, H.G., Bagchi, D., Bagchi, M., Rao, C.V.S., Dey, D.K., Satyanarayana, S., 2004a. Effects of natural extract of (-)-hydroxycitric chromium (HCA-SX) and combination of HCA-SX plus niacine-bound chromium and *Gymnema sylvestre* extract in weight loss. *Diabetes, Obesity and Metabolism* 24, 45-58.
57. Preuss, H.G., Bagchi, D., Bagchi, M., Rao, C.V.S., Dey, D.K., Satyanarayana, S., 2004b. Efficacy of a novel, natural extract of (-)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX, niacine-bound chromium and *Gymnema sylvestre* extract in weight management in human volunteers: a pilot study. *Nutrition Research* 24, 45-58.
58. Preuss, H.G., Garis, R.I., Bramble, J.D., Bagchi, D., Bagchi, M., Rao, C.V., Satyanarayana, S., 2005. Efficacy of a novel calcium/potassium salt of (-)-hydroxycitric acid in weight control. *International Journal of Clinical Pharmacology Research* 25, 133-44.
59. Ramos, R.R., Saenz, J.L., Aguilar, C.F., 1995. Extract of *Garcinia cambogia* in the control of obesity. *Investigation Medica International* 22, 97-100.
60. Rao, R., Sakariah, K., 1988. Lipid lowering and antiobesity effect of (-)-hydroxycitric acid. *Nutrition Research* 8, 209-212.
61. Rothacker, D.Q., Waitman, B.E., 1997. Effectiveness of a *Garcinia cambogia* and natural caffeine combination in weight loss: double-blind placebo-controlled pilot study. *International Journal of Obesity* 21 (Supp. 2), 53.
62. Saito, M., Ueno, M., Ogino, S., Kubo, K., Nagata, J., Takeuchi, M., 2005. High dose of *Garcinia cambogia* is effective in suppressing fat accumulation in developing male Zucker obese rats, but highly toxic to the testis. *Food and Chemical Toxicology* 43, 411-419. Comment in 2005, 43, 1683-4; Author Reply 1685-6
63. Schaller, J.L., 1999. *Garcinia cambogia* for weight loss. *JAMA* 281, 234; Author reply 235.

64. Shara, M., Ohia, S.E., Yasmin, T., Zardetto-Smith, A., Kincaid, A., Bagchi, M., Chatterjee, A., Bagchi, D., Stohs, S.J., 2003a. Dose- and time-dependent effects of a novel (-)-hydroxycitric and extract on body weight, hepatic and testicular lipid peroxidation, DNA fragmentation and histopathological data over a period of 90-days. *Molecular and Cellular Biochemistry* 254, 339-346.
65. Shara, M., Ohia, S.E., Stohs, S.J., 2003b. 90-Day oral toxicity of super CitriMax in male and female Sprague-Dawley rats. Toxicology Research Center, Creighton University Medical Center, Omaha, NE, February 21, 2003, ARC#0598, pp. 1-134.
66. Shara, M., Ohia, S.E., Schmidt, R.E., Yasmin, T., Zardetto-Smith, A., Kincaid, A., Bagchi, M., Chatterjee, A., Bagchi, D., Stohs, S.J., 2004. Physico-chemical properties of a novel (-)-hydroxycitric acid extract and its effects on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological changes over a period of 90-days. *Molecular and Cellular Biochemistry* 260, 171-186.
67. Štimac, D; Milic, S; Dintinjana, RD; Kovac, D; Ristic, S. 2002. Androgenic/Anabolic Steroid-Induced Toxic Hepatitis *Journal of Clinical Gastroenterology*. 35(4):350-352.
68. Shim, M., Saab, S., 2009, Severe Hepatotoxicity due to Hydroxycut: a case report. *Dig Dis Sci.*, 54, 406-408
69. Soni, M.G., Burdock, G.A., Preuss, H.G., Stohs, S.J., Ohia S.E., Bagchi, D., 2004. Safety assessment of (-)-hydroxycitric acid and Super CitriMax[®], a novel calcium/potassium salt. *Food and Chemical Toxicology* 42, 1513-1529.
70. Soni, et al., 2007. Expert Panel Statement. Determination of the Generally Recognized as Safe (GRAS) Status of *Garcinia cambogia* extract (CitriMax[®]) as a Flavoring Agent. Unpublished.
71. Stevens, T., Qadri, A., Zein, N. N., 2005, Two Patients with acute liver injury associated with use of the Herbal weight-loss supplement Hydroxycut. *Annals of Internal Medicine*, 142, 477-478
72. Sullivan, A.C., Triscari, J., Hamilton, J.G., Miller, O.N., 1974b. Effect of (-)-hydroxycitrate upon the accumulation of lipid in the rat. II. Appetite. *Lipids* 9, 129-134.
73. Thom, E., 1996. Hydroxycitrate (HCA) in the treatment of obesity *International Journal of Obesity and Related Metabolic Disorders* 20 (Suppl. 4), 75.
74. Thom E. 2000 A randomized, double-blind, placebo-controlled trial of a new weight-reducing agent of natural origin. *J Int Med Res* 28:229–33.
75. USDA., 2000. 1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS) (On CD-ROM). U.S. Department of Agriculture (USDA); Riverdale, Maryland. PB2000-500027 Supercedes PB98-500457
76. van Loon, L.J., van Rooijen, J.J., Niesen, B., Verhagen, H., Saris, W.H., Wagenmakers, A.J., 2000. Effects of acute (-)-hydroxycitrate supplementation on substrate metabolism at rest and during exercise in humans. *American Journal of Clinical Nutrition* 72, 1445-50.
77. Watson, J., Fang, M., Lownstein, J., 1969. Tricarballoylate and hydroxycitrate: substrate and inhibitor of ATP: citrate oxaloacetate lyase. *Archives of Biochemistry and Biophysics* 135, 209-217.

78. Watson, J.A., Lowenstein, J.M., 1970. Citrate and the conversion of carbohydrate into fat. Fatty acid synthesis by a combination of cytoplasm and mitochondria. *Journal of Biological Chemistry* 245, 5993-6002.
79. Westerterp-Plantega, M.S., Kovacs, E.M., 2002. The effect of (-)-hydroxycitrate on energy intake and satiety in overweight humans. *International Journal of Obesity and Related Metabolic Disorders* 26, 870-872.
80. WHO Newsletter #3 (2003); <http://apps.who.int/medicinedocs/en/d/Js4946e/1.4.html>
81. Xu, Z.X., Smart, D.A., Rooney, S.A., 1990. Glucocorticoid induction of fatty acid synthase mediates the stimulatory effect of the hormone on cholinephosphate cytidyltransferase activity on fetal rat lung. *Biochimica Biophysica Acta* 1044, 70-76.
82. Yeh, G, Eisenberg, D, Kaptchuk, TJ, Phillips, RS. 2003. Systematic Review of Herbs and Dietary Supplements for Glycemic Control in Diabetes *Diabetes Care* 26:1277-1294.

HEALTH HAZARD EVALUATION BOARD

The Problem: Liver toxicity following consumption of dietary supplement, Hydroxycut

HHE No.:

Background. In 2002, the Center for Food Safety and Applied Nutrition's (CFSAN) adverse event monitoring system, CAERS, began receiving reports of liver-related illnesses in persons who reported consuming the dietary supplement Hydroxycut for periods ranging from as short as a week to months. Hydroxycut has been marketed by Iovate Health Sciences, Inc (381 North Service Rd. W., Oakville, ON L6M 0H4, Canada) and manufactured by Muscletech (Blassdell, NY, USA and Mississauga, Ontario, Canada) as a weight control, fat-burner, and energy enhancement dietary supplement. Hydroxycut products bear the Iovate or Muscletech Brand names. The products contain a variety of individual ingredients as well as numerous proprietary blends such as "Hydroxagen Plus, Hydroxy Tea, HydroxyTea CF, Hydroxycut Proprietary Blend, Max! Liqui-Burn, Max! Weight-Loss Matrix, Hydroxycut Hardcore Proprietary Blend Proxyclyene, Noreidrol Intensity focus Blend, Lasidrate Delivery Blend, or Yohimbacore." The products' labels declare minerals and herbs as well as extracts from *Garcinia cambogia*, *Guarana*, *gymnema sylvestre*, *Rhodiola rosea*, and *Camellia sinensis*. Prior to 2004, Hydroxycut, contained ephedra or Ma Huang as an ingredient; however, by the beginning of 2004, Hydroxycut was ephedra-free. Subsequent to the removal of ephedra, Hydroxycut had undergone numerous formulation changes. Since the earlier formulation of Hydroxycut contained ephedra, it was generally believed that the reports of liver injury associated with the use of the product were due either to ephedra or a combination of the ingredients found in the product. However, following the removal of ephedra from Hydroxycut, CFSAN continued to receive reports of liver injury associated with the use of Hydroxycut. The present Health Hazard Evaluation describes the product in question, summarizes reports in CAERS of liver-related adverse events associated with ingestion of the product, reviews reports of Hydroxycut-associated liver toxicity published in the peer-reviewed literature, and provides a synopsis of recent communications the Center has had with hepatologists regarding the product. In addition, the HHE presents findings from CAERS reports of severe adverse events involving organ systems other than the liver.

The Product. Hydroxycut is a Registered Trademark and is part of the name of many products, examples of which include Hydroxycut, Hydroxycut Hardcore, and Hydroxycut Caffeine Free. Iovate and Muscletech, a company owned by Iovate, both market Hydroxycut products. In addition to the label-declared ingredients listed above, the products contain variable amounts of caffeine. Consumption of Hydroxycut products in amounts recommended by the label would contribute to the ingestion of approximately 600 mg of caffeine per day. By comparison (and excluding Hydroxycut ingestion), the average American consumes approximately 168 mg of caffeine per day (coffee drinkers consume approximately 280 mg caffeine per day).

The following description of Hydroxycut is provided on a web site supported by the company (<http://www.hydroxycut.com/products/hydroxycut/index.shtml> - accessed March 19, 2009):

Plain and simple, Hydroxycut was created to help you reach your weight-loss goals. This medical doctor-formulated supplement contains ingredients that are of the highest quality and have been combined to make it one of the most effective weight-loss supplements available on the market today. Hydroxycut[®] is comprised of a blend of research-proven key ingredients that can help you lose up to 4.5 times the weight than diet and exercise alone. On top of that, this top selling weight-loss supplement increases your energy and helps control your appetite too. With Hydroxycut in your diet and exercise plan, you'll be well on your way to achieving your weight-loss goals in no time!*

An example of a Hydroxycut product and its Supplement Facts is provided below.



Supplement Facts	
Serving Size 2 Rapid Release Caplets Servings Per Container 15	
Amount Per Serving	% Daily Value
Calcium (as hydroxycitrate)*** 156 mg	16%
Chromium (as polynicotinate)** 133 mcg	111%
Potassium (as hydroxycitrate)*** 218 mg	6%
Hydroxagen Plus® 1.32 g	
Garcinia cambogia extract (rind)***	†
Standardized for 60% hydroxycitric acid	
Gymnema sylvestre extract (leaf)	†
Standardized for 25% gymnemic acids	
Phosphatidylserine-enriched soy lecithin	†
Supplying 50% phosphatidylserine, 4% phosphatidylcholine, 2% phosphatidylethanolamine	
HydroxyTea® 473 mg	
Green tea extract (as <i>Camellia sinensis</i>) (leaf)	†
Standardized for 90% polyphenols, 75% catechins, 45% epigallocatechin gallate - 117 mg EGCG	
Caffeine anhydrous	†
White tea extract (as <i>Camellia sinensis</i>) (leaf)	†
Standardized for 50% polyphenols, 35% catechins, 15% EGCG	
Oolong tea extract (as <i>Camellia sinensis</i>) (leaf)	†
Standardized for 50% polyphenols, 25% catechins, 15% EGCG	
Supplying 200 mg of caffeine	
Ginger extract (as <i>Zingiber officinale</i>) (root)	†
Standardized for 5% gingerols	
Raspberry ketone	†
Quercetin dihydrate (as <i>Fava d'anta</i>)	†

†Daily Value not established.

OTHER INGREDIENTS: MICROCRYSTALLINE CELLULOSE, HYDROXYPROPYL-CELLULOSE, COATING (POLYVINYL ALCOHOL, TITANIUM DIOXIDE, POLYETHYLENE GLYCOL, TALC), SODIUM CARBOXYMETHYLCELLULOSE, CROSPVIDONE, STEARIC ACID, MAGNESIUM STEARATE, SILICA, ACESULFAME-POTASSIUM.

The directions for the product are stated as follows:

For men and women:

Take 2 caplets with a glass of water 3 times daily, approximately 30 to 60 minutes before meals (preferably before breakfast, lunch and dinner). To assess individual tolerance, refer to the chart. Do not exceed 6 caplets in a 24-hour period. Do not take within 5 hours of bedtime. For best results, use Hydroxycut for 8 weeks in conjunction with a calorie-reduced diet and a regular exercise program. Do not snack after dinner. Consume ten glasses of water per day. Read the entire label before use and follow directions.

Hydroxycut-associated liver toxicity reports in CAERS. To-date, 23 case reports of Hydroxycut-associated liver toxicity has been identified in CAERS for the period 2002 to the present. The number of reports, by event date, is listed below:

<u>Year of event</u>	<u>Number of reports</u>
2002	4
2003	3
2004	6
2005	0
2006	1
2007	6
2008	3
<u>2009</u>	<u>0</u>
Total	23

In cases for whom gender was known, 15 (65%) were female. Ages ranged from 20 years to 51 years (median = 29 years). Sixteen cases (70%) were hospitalized. The majority of cases reported no underlying risk factors for liver disease (e.g., no history of viral hepatitis, no HIV infection, no autoimmune diseases). While the reports vary in detail, several reports describe work-ups that ruled out infectious, autoimmune, and metabolic causes of liver disease. The severity of illness ranged from asymptomatic elevations in serum bilirubin to acute liver failure (one patient received a liver transplant in 2002, while a second patient was reportedly waiting for a liver transplant in 2004) to death. On March 24, 2009 CFSAN received information regarding the fatal case. The patient was a 20-year-old male who presented to an emergency room on January 19, 2007 in liver failure and hepatic encephalopathy. He was subsequently transferred to a liver transplant center where, in the operating room, he was found to have necrosis of both the large and small intestines. Given these findings, the procedure was aborted and the patient was returned to the intensive care unit. He died on February 12, 2007.

Reports of Hydroxycut-associated liver toxicity in the peer-reviewed literature. To our knowledge, there are four (1, 2, 3, 4) published reports in the peer-reviewed literature that describe liver disease that occurred in 6 persons following the consumption of Hydroxycut (Table 1). The two cases described by Stevens et al. (reference 1) were also reported to the CAERS database.

Table 1. Reports of Hydroxycut-associated liver toxicity in the peer-reviewed literature

Year	Age	Gender	Event
2005 ¹			Both cases: Previously healthy; no recent foreign travel; no sick contacts; no risk factors for viral, alcoholic, autoimmune, or hereditary liver disease. No recent use of herbal (other than Hydroxycut) or prescription medications. Both underwent a similar serologic work-up, including viral studies (hepatitis A, B, and C viruses; Epstein-Barr virus [EBV]; cytomegalovirus

	27	Male	<p>[CMV]; anti-nuclear and anti-smooth-muscle antibody levels, acetaminophen level, and toxicology screening, which was unremarkable. Both were admitted to the hospital after presenting to the emergency room.</p> <p>8-day history of fatigue and jaundice. Had been taking Hydroxycut for 5 weeks (3 tablets 3 times/day). Labs: aspartate aminotransferase [AST] 1808 (normal 15-41); alanine aminotransferase [ALT] 3131 (normal 17-63); Bilirubin 7.8 (normal 0-1.5); alkaline phosphatase 171 (normal (38-126); prothrombin time [PT] 16 seconds (10-14 seconds). Four weeks later: AST 114; ALT 304.</p>
	30	Male	<p>10-day history jaundice, fever, vomiting, fatigue. For 5 days, between the 11th and 16th days before presentation, he had been taking 9 tablets of Hydroxycut per day. Labs: AST 59; ALT 45; alkaline phosphatase 530; Bilirubin 7.8; PT 15 seconds. Abdominal CT, endoscopic retrograde cholangiogram negative. Liver biopsy: cholestasis, portal inflammation. Patient discharged after laboratory test results improved; 2 months later, both AST and ALT normal.</p>
2007 ²	19	Male	<p>Previously healthy U.S. Army soldier with no known risk factors for liver disease who, while serving in Iraq, developed acute hepatotoxicity after 4 months of ingesting Hydroxycut. Presented with nausea, vomiting, jaundice, and scleral icterus. Labs: AST 2964; ALT 1435; Bilirubin 11.7; alkaline phosphatase 153; PT 17.1 seconds. Bloodwork was negative for hepatitis A, B, C, E, as well as EBV, CMV, and HIV. Anti-nuclear antibody, anti-liver/kidney microsomal antibody, anti-smooth muscle antibody, serum acetaminophen, and urine drug screen were negative. Serum ceruloplasmin, iron studies, ferritin, and protein electrophoresis were all within normal limits. Doppler right upper quadrant ultrasound showed no gallstones and normal common bile duct caliber as well as normal portal and hepatic venous flow. The patient's jaundice resolved over the next month. His liver-associated enzymes normalized within 4 months.</p>

2008 ³	40	Female	<p>Presented with 3-day history of new-onset abdominal pain, nausea, vomiting, non-bloody diarrhea, anorexia and profound fatigue. One week prior to presentation, she began using Hydroxycut, 6 pills daily. She did not smoke or drink. Only other medication was levothyroxine for hypothyroidism. Admission labs: AST 1020; ALT 1150; Bilirubin 0.27, alkaline phosphatase 299; international normalized ration [INR] 0.96 (normal, 1.0). Diagnostic evaluation negative for hepatitis A, B,C; CMV; EBV; autoimmune liver disorders; alpha-1 anti-trypsin deficiency; and ehrlichiosis. Discharged on day 3 clinically well. As outpatient, AST 46; ALT 48. No further liver problems after 10 months of follow-up.</p>
	33	Female	<p>Prior history of pituitary adenoma, presented to ER with 1 month of new-onset jaundice, and 2-week history of nausea, abdominal pain, dark urine pruritis, and profound fatigue. During month prior to admission, took Hydroxycut for 2 weeks but discontinued supplement upon onset of symptoms. Only med= oral contraceptives; no alcohol or risk factors for viral hepatitis. Admission labs: AST 934, ALT 1570, Bilirubin 20.9; alkaline phosphatase 112; INR 1.08. Diagnostic evaluation negative for hepatitis A, B, C; CMV; Epstein-Barr virus, and herpes simplex virus infections. Autoimmune profile revealed low titer increase in anti-nuclear antibody and anti-smooth muscle antibody suggestive of immune-mediated drug-induced hepatitis. Jaundice eventually resolved and liver function normalized.</p>
2009 ⁴	28	Male	<p>Presented with 3-week history of fatigue, dyspnea on exertion, jaundice, and dark urine. Took Hydroxycut 2-3 tabs/d x 3 months prior to onset of symptoms. Also took an OTC pain-reliever containing acetaminophen 250 mg, aspirin 250 mg, and caffeine 65 mg (4 tabs/d x 10 d before onset of symptoms). History of consuming 2-3 beers/week. Labs: AST 1049; ALT 2272; alkaline phosphatase 152; Bilirubin 18.1; PT 12.8 seconds (normal 9.2-10.6 seconds); acetaminophen level undetectable. Patient's ALT and AST began to decline immediately after admission, and bilirubin peaked on hospital day #2. Viral hepatitis tests negative. Ferritin was elevated at 9519 ng/ml (normal 10-210 ng/ml). ANA, anti-smooth muscle antibody, liver kidney microsomal antibody and soluble liver antigen antibody were all negative. Despite normal serum copper, 24-hour urine copper level was elevated at 290 mcg/dl (normal 3-50 mcg/dl). Slit-lamp for Kaiser-Fleischer rings equivocal. Investigators attributed elevated urine copper to cholestasis, not to Wilson's disease, and stated: "his presentation was most consistent with hepatotoxicity with</p>

			Hydroxycut.”
--	--	--	--------------

The aforementioned cases are consistent with the diagnosis of idiosyncratic hepatotoxicity for a number of reasons: the temporal relationship between the consumption of Hydroxycut and the development of acute liver injury in persons who had no history of known liver disease; the exclusion of other causes of liver disease following extensive work-ups; and the resolution of liver injury upon discontinuation of Hydroxycut; the development of liver injury is not dose- dependent. Also apparent were two distinct patterns of liver injury: cholestatic and necrotic. It is not unusual for a single herbal preparation to produce more than 1 type of clinicopathologic liver injury (5).

Discussions with hepatologists. In discussions in March and April of 2009 with hepatologists Tse-Ling Fong, M.D. of the University of Southern California and William Lee, M.D. of the University of Texas Southwestern Medical Center, CFSAN has become aware of these physicians’ case series of patients with severe liver disease associated with the use of Hydroxycut. Two cases from this series, representing additional cases to the ones reported to CFSAN, underwent liver transplantation following acute liver failure.

Serious non-hepatic adverse events identified in the CAERS database or the literature. When the CAERS database was queried for other serious adverse events associated with Hydroxycut, cases of seizures, rhabdomyolysis, and cardiovascular disorders were identified. For example, from 2004 to 2008, the CAERS database received 4 case reports describing consumers who experienced a seizure following ingestion of Hydroxycut. In one instance, a 26-year-old consumer increased her daily intake of Hydroxycut from 2 to 4 caplets on December 6, 2008. At 2 p.m. that day, following ingestion of the second serving of 2 caplets, the consumer felt tired and lay down. She was found by another person to be having a “seizure” (shaking and drooling).

The consumer was taken to the emergency room where a physician told her to discontinue using Hydroxycut.

The case report describing rhabdomyolysis involved a 23-year-old male who had been consuming Hydroxycut on and off over an eight-month period in 2002. On the day of hospital admission, he had taken 2 tablets for energy prior to working out. He reported feeling nausea, and then several hours later, he had severe shoulder pain and dark urine. He was diagnosed as having rhabdomyolysis on admission to the hospital. In addition to this CAERS report, the Board is aware of one case of Hydroxycut-associated rhabdomyolysis reported in the peer-reviewed literature. In this report, Dehoney and Wellen (6) described an 18-year-old male who experienced rhabdomyolysis after consuming Hydroxycut as per the product's instructions. During his overnight hospitalization, he received 6 liters of fluid before discharge.

The Board also identified 46 reports in CAERS of Hydroxycut-associated cardiovascular adverse events. These events ranged in severity from palpitations to a heart attack. Nineteen of these reports were received during or after 2004, a period when Hydroxycut's formulation was believed to be free of ephedra.

Conclusion:

Three lines of evidence derived from multiple disparate sources suggest it is very likely that exposure to Hydroxycut can cause idiosyncratic hepatotoxicity. First, many of the subjects described in the adverse event reports to CAERS, in the peer-reviewed literature, and in the case series described by hepatologists reported no history of liver disease or risk factors for liver disease (e.g., alcohol consumption, previous viral infection, hereditary factors, etc.) prior to experiencing liver injury following the ingestion of Hydroxycut. Second, in many subjects, thorough diagnostic evaluations performed in multiple settings ruled out a number of known causes of liver disease, including viral hepatitis, autoimmune diseases, and metabolic/inherited disorders. Third, prompt resolution of liver disease occurred in a number of patients following cessation of Hydroxycut ingestion. Further, while some adverse event reports involved users who had consumed more than the daily dosage recommended on the products' labeling, if these reports were excluded from consideration, the remaining evidence demonstrates liver-related adverse effects following exposure to Hydroxycut. In addition to Hydroxycut-associated liver-related adverse effects, the Board is aware of a number of CAERS reports that describe seizures, rhabdomyolysis, and cardiovascular signs and symptoms.

The Board does not know what ingredient(s) of Hydroxycut are responsible for producing liver toxicity. In addition, there is insufficient information to determine whether there is a dose-response effect between Hydroxycut ingestion and liver disease or whether its effects are cumulative over time. However, based on the totality of evidence presented above, the Board concludes that the ingestion of the dietary supplement, Hydroxycut, presents a severe potentially life-threatening hazard to some users. Although Hydroxycut-induced hepatotoxicity has been reversible in most patients that have come to the attention of CFSAN, in certain instances acute liver failure has resulted that has

required liver transplantation to ensure survival and death occurred in one instance prior to transplantation.

Members Present

Robert Mozersky, D.O.

Karl Klontz, MD

Linda M. Katz, M.D., M.P.H.
Interim Chief Medical Officer

References

1. Stevens T, Qadri A, Zein NN. Two patients with acute liver injury associated with use of the herbal weight-loss supplement Hydroxycut. *Ann Intern Med* 2005;142:477-8.
2. Jones FJ, Andrews AH. Acute liver injury associated with the herbal supplement Hydroxycut in a soldier deployed to Iraq. *Am J Gastroenterol* 2007;102:2357.
3. Dara L, Hewett J, Lim JK. Hydroxycut hepatotoxicity: A case series and review of liver toxicity from herbal weight loss supplements. *World J Gastroenterol* 2008;14:6999-7004
4. Shim M, Saab S. Severe hepatotoxicity due to Hydroxycut: a case report. *Dig Dis Sci.* 2009 Feb;54(2):406-8. Epub 2008 Jul 26.
5. Miller SC. Safety concerns regarding ephedrine-type alkaloid-containing dietary supplements. *Mil Med* 2004;169:87-93
6. Dehoney S, Wellein M. Rhabdomyolysis associated with the nutritional supplement Hydroxycut. *Am J Health Syst Pharm* 2009 Jan 15;66(2):142-86.